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**Linking Estrogens, Prostatitis and Prostate Cancer**

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This study aims to examine the role that estrogens and inflammation may play in the development and progression of prostate cancer. In order to demonstrate this, we have sought to characterise the inflammation and potential development of pre-malignancy in the aromatase over-expressing (AROM+) mouse as well as examine the impact of inflammation on aromatase expression and estrogen metabolism in human tissue.

Significant progress has been made towards the aims. We have demonstrated that the AROM+ mouse develops chronic inflammation from 30 weeks of age and this inflammation has been extensively and thoroughly characterised; these data have also indicated a novel and significant role for mast cells in this process. We have also demonstrated that the AROM+ mice also develop pre-malignant lesions by 52 weeks of age, which is after the emergence of inflammation. These lesions have also been thoroughly characterised.

Taken together, these data demonstrate that exposure to elevated physiological levels of estrogens are associated with the development of prostatic inflammation, and, subsequently, pre-malignancy. Additionally, these early data show that the AROM+ mouse is a novel, non-bacterial model for the study of prostate inflammation.

**15. SUBJECT TERMS**
estrogen, prostate, prostatitis, inflammation, cancer

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**INTRODUCTION**

Prostatitis affects ~10% of men and prostate cancer (PCa) is a leading cause of death in Western men. Significantly, inflammation has been associated with the development of carcinoma in a number of tissues, including the prostate (*1*). However, despite its prevalence and putative role in PCa, prostatitis remains poorly understood and the vast majority of cases (~ 90–95%) are of unknown aetiology. Estrogens can cause prostatic inflammation and it is also significant that estrogens have been adversely implicated the development of PCa (*2*). Consequently, the overall aim of this study is to demonstrate that estrogens induce inflammation which leads to pre-malignancy and malignancy of the prostate gland. Ultimately, this study will establish the aromatase over-expressing (AROM+) mouse as a novel, non-bacterial model for the study of prostatitis. Furthermore, by linking estrogens, prostatitis and prostate cancer, we can provide better treatment for prostatitis, thus potentially also preventing the progression to PCa. Lastly, by defining the role of estrogens and inflammation in PCa this study will also stimulate investigation into new methods for the detection, prevention and treatment of PCa.

**BODY**

**Task 1: To characterise and quantify the inflammation in AROM+ mice, demonstrating that this is an estrogen-induced model of prostatitis. [Years 0-1].**

**Overview:**

Our preliminary data show that inflammation develops in aged AROM+ mice. This model may provide a novel means to examine the effect of increased endogenous estrogens within a physiological setting, specifically in relation to the development of inflammation and malignancy. We aim to thoroughly characterise and quantify the inflammation in AROM+ mice, thus demonstrating that this is an estrogen-induced model of non-bacterial prostatitis.

**Progress:**

This task has been completed. We have examined the prostates of AROM+ and wt mice at a variety of ages to determine the onset of, as well as to characterise and quantitate the inflammation.

Initially, the levels of testosterone (T) and estradiol (E) in AROM+ mice compared to their wild-type littermate controls was determined at specified ages throughout life. This data clearly demonstrates that the AROM+ mice have significantly increased levels of estrogen, and reduced levels of testosterone, throughout life (Figure 1).

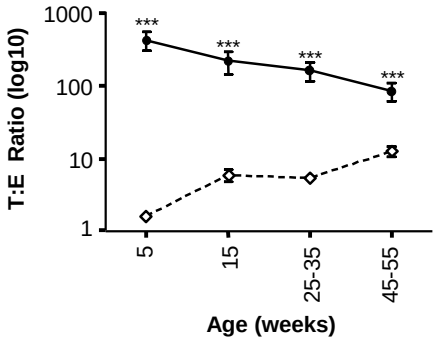


**Figure 1: Serum Testosterone and Estradiol Levels in AROM+ and wt Mice**

Temporal analysis of the testosterone and estradiol levels in AROM+ and wt mice, as determined by RIA. The serum testosterone:estradiol ratio was found to be significantly smaller in AROM+ animals versus wt at all ages. (◊ = AROM+; • = wt; significance denoted by asterisks, *** < 0.001; n ≥ 5 for each group).

In conjunction with the hormone levels, we have also quantitated the expression of the hormone receptors, ERα, ERβ and AR, in AROM+ and wt tissues. No significant changes in ERβ and AR were observed, but, consistent with the predicted role of ERα in inflammation, ERα levels were found to be significantly elevated in the AROM+ tissues throughout life (Figure 2).

| Genotype | % Epithelial Cells ERα Positive | Significance |
|---|---|---|
| wt | 0.8 +/- 0.5 | |
| AROM+ | 14.1 +/- 7.1 | * (P = 0.0463) |

**Figure 2: Local aromatase over-expression increases ERα expression**

ERα expression in intact wt and AROM+ VP tissue was quantitated using stereology and was significantly increased in intact AROM+ tissues when compared with wt controls (representative data from 15w old AROM+ and wt animals; significance denoted by asterisks, * < 0.05).

Late in life (40w+) the AROM+ mice develop chronic inflammation of the prostate (Figure 3, Representative Images). The temporal emergence of this inflammation was documented, and it was found that there was a significant increase in mast cell numbers immediately following puberty that persisted throughout life. The emergence of the more chronic inflammation was found to occur from 30 weeks of age and progressively increased in incidence and severity with increasing age, becoming highly significant by 40 weeks of age (Supporting Data, Figure 1).

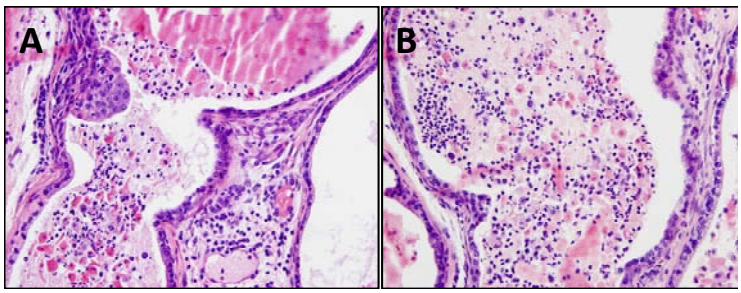


**Figure 3: Chronic Inflammation Develops in the AROM+ Prostate with Aging**
Chronic inflammation developed by 40 weeks of age in the AROM+ prostate and was characterised by an abundance of mononuclear lymphocyte-like cells with a few granulocytes within the stroma (A) along with a more mixed population of cells within the lumen, including macrophages, plasma cells, neutrophils and some cellular debris (A and B).

This inflammation was characterised and examined in detail in order to determine the type, range and number of specific leukocytes responsible. This was performed using a combination of immunohistochemistry and stereology and revealed a significant increase in mast cell numbers as well as significant increases in neutrophils, T-lymphocytes and macrophages. B lymphocyte numbers, however, remained unaltered (Supporting Data, Figure 1).

In order to identify some of the potential mechanisms arising from the inflammation that may be mediating the development of malignancy, the expression of a number of key inflammatory cytokines and chemokines in the AROM+ prostate was examined. This analysis revealed significant changes to the expression of a number of cytokines, chemokines and their receptors (Supporting Data, Figure 2). Of particular significance were significant increases in the expression of CCL20, CCL8, CCR6, CCR5 and CCR2, all of which have previously been implicated in the development of PCa (*3-5*).

A further additional finding of this work has been the documentation of a previously unreported pathology in the AROM+ animals, specifically, the development of scrotal hernias. This pathology has been found to occur in aging animals with increasing incidence and severity with age (Supporting Data, Figure 3). At present this does not appear to adversely affect the animals overall health, however, the significance of this finding remains to be determined.

**Outcome(s):**
Overall, these data demonstrate that physiologically elevated levels of estrogens are associated with the development of prostatitis. Specifically, this work has identified the age of onset of inflammation, as well as the type and extent of the inflammation, in AROM+ mice. This provides baseline information critical for subsequent experiments. This work also implicates a role for estrogens and, specifically, ER α, in the onset of prostatitis and also reveals potential mechanisms that may lead to the development of malignancy with alterations to a number of key cytokines and chemokines. Finally, and most significantly, these data also establish the AROM+ mouse as a model of non-bacterial prostatitis.

**Task 2: To demonstrate that the AROM+ inflammation is due to activation of ERα and can be blocked by the administration of a selective ERα antagonist. [Years 1-2].**

**Overview:**

ERα is up-regulated and essential for exogenous estrogen-induced inflammation (*6*). Consequently, we predict that the inflammation in AROM+ mice will correlate with increased levels of systemic or intra-prostatic estrogens and ERα expression. We also predict that blocking ERα (but not ERβ) will abrogate and/or prevent the inflammation. This will be tested by using a selective ERα antagonist.

**Progress:**

Experimental groups of wt and AROM+ mice are currently being established and aged immediately prior to the onset of inflammation (30w) or after chronic inflammation is apparent (40w). They will subsequently be treated with the ERα antagonist to determine if this compound prevents the onset of the inflammation, or reduces and/or abrogates the inflammation.

This work is currently in progress, with experimental groups currently being aged prior to treatment.

**Outcome(s):**

No data has been generated to date as experimental groups are being established, treated and aged. Ultimately, this work will demonstrate the role of ERα in prostate inflammation. If the ERα antagonist be able to reduce and/or eliminate the prostatic inflammation this will indicate the potential of such compounds in the treatment of prostatitis.

**Task 3: To confirm the specific role of ERα by demonstrating that a selective ERβ agonist fails to block inflammation. [Years 1-2].**

**Overview:**
ERβ has a putative anti-inflammatory role in a number of diseases such as inflammatory bowel disease and blood cystitis. Currently it is not known whether ERβ plays a similar role in the prostate. We will test the role of ERβ by examining whether an ERβ agonist prevents or abrogates the development of inflammation in AROM+ mice.

**Progress:**

Experimental groups of wt and AROM+ mice are currently being established and aged immediately prior to the onset of inflammation (30w). They will subsequently be treated with the selective ERβ agonist to determine if the agonist prevents the onset of the inflammation.

This work is currently in progress, with experimental groups currently being aged prior to treatment.

**Outcome(s):**
No data has been generated to date as experimental groups are being established, treated and aged. However, we do not predict a local role for ERβ in inflammation. We anticipate that the ERβ agonist will not block inflammation, supporting the concept that ERα is the receptor subtype driving prostatic inflammation.

**Task 4: Determine if premalignant lesions and/or frank malignancy occur in AROM+ mice. [Years 0.5-1.5].**

**Overview:**

We will determine if pre-malignant and malignant pathologies occur in AROM+ mice of an age when chronic inflammation becomes evident. Our approach is to detect and quantify the development of premalignant lesions (prostatic intraepithelial neoplasia; PIN) or frank malignancy by examining the tissue pathology, natural history of neoplastic progression and the expression of a number of molecular markers.

**Progress:**

Although this task is still in progress, the majority of work has been completed.

We have examined the prostate tissues of AROM+ mice following the emergence of inflammation and examined the development of pre-malignant lesions. To date we have successfully demonstrated the emergence of PIN lesions in the AROM+ mice (Figure 4), but have found no evidence of frank or invasive carcinoma.

These lesions were examined, classified and characterised using immunohistochemistry and the pathological criteria set out in the MMHCC Prostate Pathology Committee's Bar Harbour classification system for mouse prostate pathology (7).

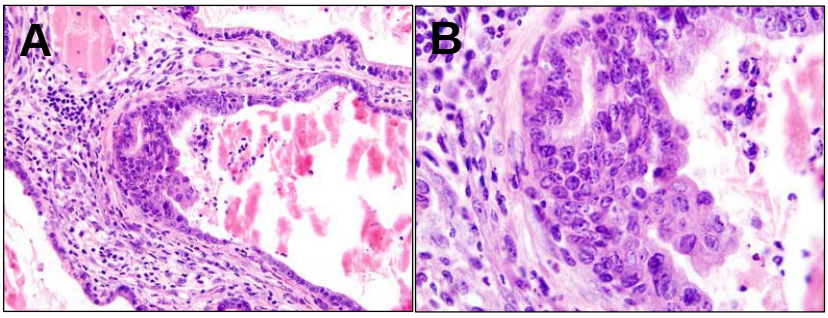


**Figure 4: Representative H&E micrographs of a pre-malignant PIN lesion in the AROM+ prostate. (A)** Low power image demonstrating the lesion and associated inflammation throughout the stroma and in the lumen proximal to the lesion. **(B)** Higher power image demonstrating the key features of PIN: stratified abnormal epithelial cells, some evidence of tufting, enlarged atypical nuclei and highly prominent nucleoli.

No frank malignancy and/or invasive carcinoma have been demonstrated in AROM+ tissues to date, although mice are being aged further and work is still underway to examine this.

**Outcome(s):**

To date, we have demonstrated that AROM+ mice develop PIN lesions following the emergence of chronic inflammation. This outcome, therefore, links inflammation with the occurrence of premalignant lesions.

**Task 5 : Determine if there is increased susceptibility to hormonally induced malignancy in AROM+ mice [Years 0.5-2].**

**Overview:**
Mice do not spontaneously develop PCa, and it is possible that the AROM+ mice may only show premalignant changes without any evidence of frank malignancy. In order to determine whether they are more susceptible to malignancy, we will test their response to combinatorial hormone-induced carcinogenesis when compared to wild-type controls.

**Progress:**
Experimental groups of wt and AROM+ mice have been established and treated with testosterone propionate (T) and/ or 17 α-estradiol benzoate (E). The specific treatment groups are: T+E, T alone, E alone, empty implant and untreated.

Each group will be treated for 4 months, after which the prostate lobes from all mice will be collected and examined to identify and quantify the pathologies present and to confirm molecular changes associated with malignancy.

This work is currently in progress, with experimental animals currently undergoing treatment after having been aged.

**Outcome(s):**
No data has been generated to date as experimental groups are being established, treated and aged. However, we predict that this work will demonstrate whether the AROM+ mice are more susceptible to hormonally induced malignancy than their wild-type littermate controls. This will potentially provide the first direct evidence linking physiologic estrogen up- regulation and prostate malignancy via inflammation.

**Task 6: Determine whether aromatase expression in human tissues is altered with prostatitis [Years 0-2].**

**Overview:**
We have previously demonstrated that aromatase expression and regulation is altered with PCa (*8*). Additionally, as the aromatase promoter used documented in PCa indicates that these are responsive to pro-inflammatory cytokines it is likely that prostatitis and inflammation may play a role in driving aberrant aromatase expression. The onset and role of these changes in the development and progression of PCa have yet to be studied. Consequently, we aim to determine whether aromatase expression in human tissues is altered with prostatitis.

**Progress:**
We have obtained a number of archival human tissue specimens in which the uropathologist has identified chronic inflammation. To date, we have successfully utilised laser capture micro-dissection to isolate regions of benign and malignant epithelia, as well as epithelia and tumour cells immediately adjacent to inflammatory cells.

However, we have been unable to assess the aromatase expression and promoter utilisation in these samples due to poor RNA recovery from the archival samples. This has been attributed to the tissue fixation protocol that is routinely used by the pathology laboratory as control samples prepared from frozen tissues have been successful and have yielded high quality RNA in sufficient quantity for analysis.

This work is still in progress. Alternative sources of tissue are being investigated (ideally frozen tissues), as are alternative methods for the extraction and purification of RNA from the archival tissue samples.

**Outcome(s):**
To date, there are no outcomes for this task. Ultimately, however, we predict that in regions of prostatic inflammation epithelial cells will show altered aromatase expression, indicative of aberrant local estrogen synthesis.

**KEY RESEARCH ACCOMPLISHMENTS**

List of key research accomplishments arising from this research:

- Demonstration of the AROM+ mouse as a model of non-bacterial prostatitis
  - Characterisation of the inflammation in AROM+ mice
    - Immunohistochemical and stereological quantitation of leukocytes at various ages
      - Significant increase in mast cells from puberty
      - Significant increases in T-lymphocytes, macrophages and neutrophils with chronic inflammation
  - Identification of expression of key chemokines and cytokines involved in the AROM+ inflammation
- Immunohistological and stereological characterisation of AROM+ tissues
  - Significant increase in ERα expression
- Demonstration of hormone profiles in AROM+ mice
  - Reduced serum Testosterone:Estrogen ratio throughout life
- Demonstration of the development of pre-malignant (PIN) lesions in AROM+ mice
  - Immunohistological characterisation of pre-malignant lesions

**REPORTABLE OUTCOMES**

Reportable outcomes that have resulted from this research (to date):

**1. Manuscripts:**
A.
Stuart J. Ellem, Hong Wang, Matti Poutanen and Gail P. Risbridger.
"Increased endogenous estrogen synthesis leads to the sequential induction of prostatic inflammation (prostatitis) and prostatic pre-malignancy."
Am. J. Pathol. (In Review)

**2. Abstracts / Presentations:**
**A.**
Invited Symposia Presentation (Refer Appendix 1A for abstract)
Stuart J. Ellem, Hong Wang, and Gail P. Risbridger
"Hormones, Prostatitis and Prostate Cancer"
4th Annual Australian Health & Medical Research Congress
November 2008
Brisbane, Australia

**B.**
Poster Presentation (Refer Appendix 1B for abstract)
Stuart J. Ellem, Hong Wang, Matti Poutanen and Gail P. Risbridger
"Sequential induction of prostatic inflammation (prostatitis) and prostatic malignancy due to increased endogenous estrogen synthesis"
9th International Aromatase Conference
October 2008
Shanghai, China

**3. Patents & licenses: Nil**
**4. Degrees obtained: nil**
**5. Development of cell lines: Nil**
**6. Tissue or serum repositories: Nil**
**7. Informatics such as databases and animal models: Nil**
**8. Funding applied for based on this award: Nil**
**9. Employment or research opportunities: Nil**

**CONCLUSION**

In summary, the data generated from this study to date demonstrate that exposure to elevated physiological levels of estrogens is associated with the development of prostatic inflammation, and, subsequently, pre-malignancy. These data also show that the AROM+ mouse is a novel, non-bacterial model for the study of prostate inflammation.

This study has already generated some highly novel and significant data. Foremost of these is the establishment of the AROM+ mouse as model of non-bacterial prostatitis, fulfilling a key need that was stressed in the report from the Bar Harbour Consensus meeting (7). In addition to this, this study has also demonstrated a potential role for mast cells in prostatitis and has identified key cytokines and chemokines involved in the inflammatory process induced by estrogens.

Further work is currently underway to examine some of the underlying mechanisms involved in this process. This work will identify any pivotal roles of the estrogen receptors, ERα and ERβ, using specific agonists and antagonists. Further work that is being undertaken will, once completed, provide further insight in to how estrogenic exposure and metabolism is related to inflammation and how this predisposes to, or increases the risk of, developing PCa.

Therefore, at the conclusion of this investigation, this study will have provided significant new insight into the role of estrogen in prostatic inflammation, and, subsequently, the role of inflammation in the development of PCa. Ultimately, the data generated from this study may provide the basis of new therapeutic targets for the treatment or prevention of prostatitis and PCa.

**REFERENCES**

1. A. M. De Marzo *et al.*, *Nat Rev Cancer* **7**, 256 (Apr, 2007).
2. S. J. Ellem, G. P. Risbridger, *Nat Rev Cancer* **7**, 621 (Aug, 2007).
3. P. Ghadjar *et al.*, *J Cancer Res Clin Oncol* **134**, 1181 (Nov, 2008).
4. Y. Lu *et al.*, *J Cell Biochem* **101**, 676 (Jun 1, 2007).
5. G. G. Vaday, D. M. Peehl, P. A. Kadam, D. M. Lawrence, *Prostate* **66**, 124 (Feb 1, 2006).
6. W. A. Ricke *et al.*, *FASEB J* **22**, 1512 (May, 2008).
7. S. B. Shappell *et al.*, *Cancer Res* **64**, 2270 (Mar 15, 2004).
8. S. J. Ellem, J. F. Schmitt, J. S. Pedersen, M. Frydenberg, G. P. Risbridger, *J Clin Endocrinol Metab* **89**, 2434 (May, 2004).

**APPENDICES**

**APPENDIX 1A**

**Invited Symposia Presentation given by Stuart J Ellem at the 4th Annual Australian Health & Medical Research Congress (November 2008, Brisbane, Australia).**

**Hormones, Prostatitis and Prostate Cancer**

**Stuart J. Ellem, Hong Wang, and Gail P. Risbridger**
Centre for Urological Research, Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia.

Prostatitis (inflammation of the prostate) is the most common prostatic condition worldwide and afflicts men of all ages. Significantly, prostatitis is believed to contribute and lead to the development and/or progression of prostate cancer (PCa), similar to the role that inflammation plays in the development of malignancy in other organs. Despite this, the underlying causes of prostatitis remain unknown, with 90-95% of all prostatitis cases having no evidence of infection and an unknown aetiology.

Several causes of prostatitis have been postulated, however, of particular interest is a link between estrogen exposure and the development of prostatic inflammation. This link has emerged from over ten years of research from various laboratories, particularly our own. The data that has been obtained from these studies show that the prostate gland is particularly sensitive to estrogen during development in foetal and neonatal life; transient estrogen exposure before puberty results in inflammation later in life, well after the exposure has occurred. This research has also demonstrated that this action is mediated by ER $\alpha$ and involves a cascade of events that permanently and irreversibly alters gene expression patterns in the gland.

Inflammation is also linked to cancer, with ~20% of all human cancers arising due to chronic inflammation. This is also believed to be true for prostatitis and there is a growing body of evidence implicating a role for inflammation in the aetiology of PCa. Previous studies using a number of animal models have demonstrated that estrogen is also implicated in the development of PCa (in addition to androgens), again via ER$\alpha$.

Significantly, our recent studies using the aromatase over-expressing mouse (that has elevated systemic estrogens; AROM+) show that chronic inflammation develops in these animals with age, followed by the emergence of pre-malignant lesions. This progressive onset of prostatitis and pre-malignant lesions reveals a link between estrogens, inflammation and malignancy in the prostate.

The identification of estrogen as a cause of prostatitis, as well as a factor in the development of PCa, is significant and may lead to the development of new and better treatments for both of these diseases. Additionally, our work demonstrates that the AROM+ mouse is a novel, non-bacterial model for the study of prostate inflammation that has the potential to yield important new insights into this disease.

**APPENDIX 1B**

**Poster Presentation given by Stuart J Ellem at the 9th International Aromatase Conference (October 2008, Shanghai, China).**

**Sequential induction of prostatic inflammation (prostatitis) and prostatic malignancy due to increased endogenous estrogen synthesis.**

**Ellem SJ[1], Wang H[1], Poutanen M[2], Risbridger GP[1]**
[1] Centre for Urological Research, Monash Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia.
[2] Department of Physiology, University of Turku, Turku, Finland.

Prostatitis is a condition that causes substantial morbidity to male sufferers through the associated constellation of urinary symptoms, sexual dysfunction and pelvic pain and 90-95% of cases are of unknown aetiology. Significantly, inflammation is associated with the development of carcinoma in a number of tissues. This is also true of the prostate, with chronic inflammation (prostatitis) being associated with premalignant and malignant lesions. The causes and origins of prostatitis represent a significant gap in our knowledge and the development of new animal models is imperative to improve our understanding this disease and its role in the development of prostate cancer (PCa).

Estrogens cause inflammation in the prostate and in this study we examine the murine prostatic phenotype induced by elevated estrogen levels due to aromatase enzyme over-expression (AROM+ mouse). We show that although the early-life development of the prostate in these mice is normal, there are progressive changes during life that culminate in the development of pre-malignant lesions. Growth of the gland is reduced at puberty and there is a significant elevation in mast cell numbers. Upon further aging, chronic inflammation becomes evident by 40 weeks of age with increased mast cell, macrophage and lymphocyte infiltrates. Array analysis also revealed significant changes in the expression of a number of inflammatory mediators. Finally, pre-malignant PIN-like lesions developed by 52 weeks of age.

These data demonstrate that exposure to elevated physiological levels of estrogens are associated with the development of prostatic inflammation, and, subsequently, pre-malignancy. Additionally, these data show that the AROM+ mouse is a novel, non-bacterial model for the study of prostate inflammation. Overall, this study links estrogens to inflammation and pre-malignancy of the prostate, further implicating estrogen in the induction and/or progression of prostate cancer.

**SUPPORTING DATA**

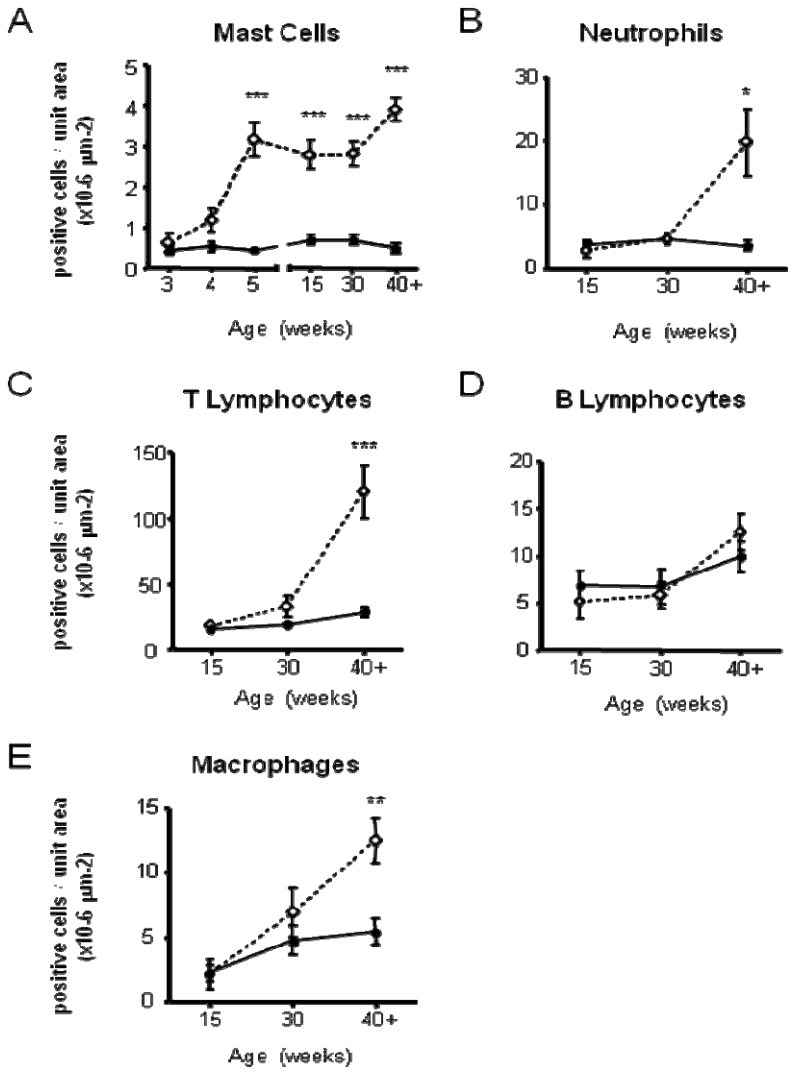


**FIGURE 7: Quantitation and Characterisation of the Inflammatory Infiltrate in the AROM+ Prostate**

Tissue sections were stained using specific stains for a mast cells (toluidine blue), neutrophils (ly6g/Gr1), T lymphocytes (CD3), B lymphocytes (B220/CD45R) or macrophages (F4- 80) and then quantitated using systematic random sampling throughout the tissue and stereology. Significant increases in mast cell numbers were apparent immediately following puberty and persisted throughout life (A). Significant increases in neutrophils, T lymphocytes and macrophages were evident from 40 weeks of age (B, C & E) while B lymphocyte levels remained unaltered (D) (n ≥ 5 per genotype per age per marker; ◊ = AROM+; • = wt; significance denoted by asterisks, * < 0.05, ** < 0.01, *** < 0.001).

| Gene | Name | Fold Change | P Value | Fold Up - or Down- Reg |
|---|---|---|---|---|
| **BCL6** | B-cell lymphoma 6 protein | 0.46 | 0.008094 | -2.18 |
| **C3** | Complement component 3 | 4.03 | 0.020398 | 4.03 |
| **Casp1** | Caspase 1 | 2.33 | 0.009186 | 2.33 |
| **CCL12** | Chemokine (C-C motif) ligand 12 | 11.19 | 0.002889 | 11.19 |
| **CCL17** | Chemokine (C-C motif) ligand 17 | 2.98 | 0.026750 | 2.98 |
| **CCL19** | Chemokine (C-C motif) ligand 19 | 3.4 | 0.027870 | 3.4 |
| **CCL20** | Chemokine (C-C motif) ligand 20 | 25.42 | 0.000015 | 25.42 |
| **CCL8** | Chemokine (C-C motif) ligand 8 | 10.05 | 0.003893 | 10.05 |
| **CCL9** | Chemokine (C-C motif) ligand 9 | 2.39 | 0.048203 | 2.39 |
| **CCR2** | Chemokine (C-C motif) receptor 2 | 3.81 | 0.020781 | 3.81 |
| **CCR3** | Chemokine (C-C motif) receptor 3 | 3.08 | 0.029387 | 3.08 |
| **CCR4** | Chemokine (C-C motif) receptor 4 | 3.59 | 0.047536 | 3.59 |
| **CCR5** | Chemokine (C-C motif) receptor 5 | 2.8 | 0.030673 | 2.8 |
| **CCR6** | Chemokine (C-C motif) receptor 6 | 4.31 | 0.028034 | 4.31 |
| **CD40LG** | CD40 ligand | 3.58 | 0.026211 | 3.58 |
| **CXCL1** | Chemokine (C-X-C motif) ligand 1 | 0.21 | 0.019112 | -4.74 |
| **CXCL9** | Chemokine (C-X-C motif) ligand 9 | 2.37 | 0.046222 | 2.37 |
| **CXCR5** | Chemokine (C-X-C motif) receptor 5 | 4.31 | 0.044996 | 4.31 |
| **IL-10** | Interleukin 10 | 5.23 | 0.009659 | 5.23 |
| **IL-10Ra** | Interleukin 10 receptor, alpha subunit | 2 | 0.007194 | 2 |
| **IL-1a** | Interleukin 1, alpha | 0.5 | 0.019392 | -2 |
| **IL-1R2** | Interleukin 1 receptor, type II | 3.9 | 0.007045 | 3.9 |
| **IL-2Rg** | Interleukin 2 receptor, gamma | 3.13 | 0.030638 | 3.13 |
| **ITGB2** | Integrin beta 2 | 2.74 | 0.037346 | 2.74 |
| **SPP1** | Secreted phosphoprotein 1 | 4.14 | 0.016364 | 4.14 |
| **XCR1** | Chemokine (X-C motif) receptor 1 | 3.71 | 0.010119 | 3.71 |

**Figure 2: The Expression of Inflammatory Cytokines and Chemokines is Altered in AROM+ Mice**

Relative expression of inflammatory cytokines and chemokines in the inflamed AROM+ prostate as compared to wt controls. Only results with a relative mRNA expression at least two-fold higher or lower than that of the *wt* control mice and a statistical P value < 0.05 were considered significant. Of particular note are the increases in CCL20, CCL8, CCR2, CCR5 and CCR6, all of which have previously been implicated in the development of PCa.

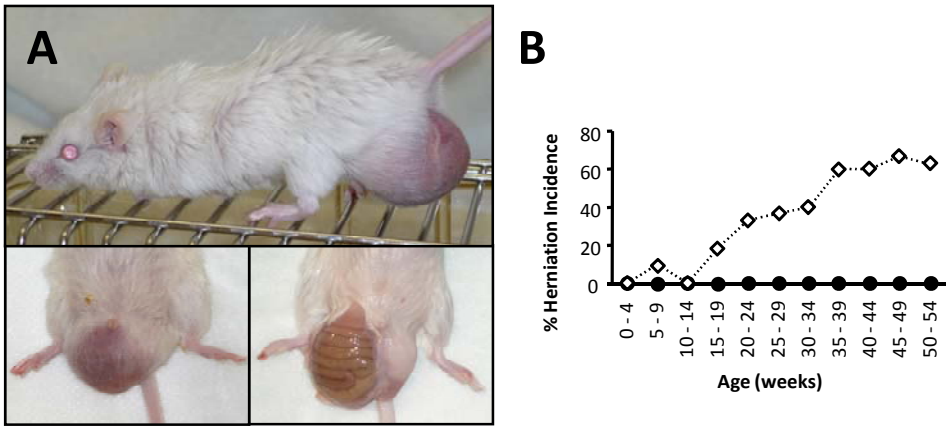


**Figure 3: The Development of Scrotal Hernias in AROM+ Mice with Aging.**
The AROM+ mice were found to spontaneously develop scrotal hernias (A; examples from a severe hernia), that increased in incidence and severity with age (B; n ≥ 10 per genotype per age; ◊ = AROM+; • = wt).